Toluene-3,4-Dithiol Analysis of Blood for Assessing Carbon Disulfide Exposure

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Carbon disulfide is a neurotoxic compound used in the production of viscose rayon, and is a major decomposition product of dithiocarbamates used in agriculture, industry, and medicine. Methods used currently for assessing exposure to CS₂ are limited in their ability to evaluate cumulative exposures and provide useful information for relatively short periods of time after exposure has ended. The present investigation evaluates a method for monitoring CS₂ exposure that consists of cleaving the thiocarbonyl function of free CS₂ or certain CS₂-generated modifications on proteins using toluene-3,4-dithiol. The resulting toluene trithiocarbonate product is then quantified using reverse-phase high-performance liquid chromatography. The sensitivity, dose response, kinetics and specificity of this biomarker in blood were examined in rats administered CS₂ by inhalation, intraperitoneal injection, or gavage for acute through subchronic periods. Dithiol reactive functions in plasma and hemolysate demonstrated a linear dose response over a wide range of exposure levels, were dependent upon the duration of exposure, and appeared to have an appropriate sensitivity for evaluating occupational levels of exposure. Elimination rates of dithiol reactive functions may also be dependent upon exposure duration and exhibit different kinetics for plasma and hemolysate suggesting that elimination rates may be useful for estimating cumulative exposure and intervals between exposure and sample procurement. Dithiol analysis, used in conjunction with previously established erythrocyte protein cross-linking biomarkers, may provide a means to characterize the internal dose of CS₂ resulting from acute through chronic periods, and may provide insight into the level of CS₂-mediated covalent protein modifications occurring within the nervous system.

Key Words: carbon disulfide; toluene-3,4-dithiol; biomarker; tri-thiocarbonate; dithiocarbamates.

Human exposure to CS₂ may occur directly from its use as a solvent in the production of rayon or indirectly from the decomposition of dithiocarbamates and bis(thiocarbamoyl)dissulfides that are used in agriculture, industry, and medicine. Neurotoxicity associated with CS₂ exposure is well documented, requiring that individuals at risk be monitored for internal exposure (Chu et al., 1995, 1996; Huang et al., 1996; Ruijten et al., 1993). Because the volatility and reactivity of CS₂ complicates its direct measurement in biological samples, the urinary metabolite 2-thiothiazolidine-4-carboxylic acid (TTCA) is presently the most widely used biomarker of exposure; however, there are limitations of this biomarker. Evidence has been presented for non-CS₂ dietary sources of TTCA (Simon et al., 1994) and a greater percent of the internal dose of captan is biotransformed to TTCA than is CS₂ (DeBaun et al., 1974). Additionally, background peaks interfere with the HPLC analysis of TTCA when evaluating low level exposures (Cox and Que Hee, 1996). In addition, the relatively short elimination half-life for TTCA (Campbell et al., 1985; Cox et al., 1996) restricts the period following cessation of exposure during which useful samples can be obtained. Probably, the most significant limitation results from the inability to derive information pertaining to the duration of exposure, and it has been suggested that urinary excretion of TTCA may even decrease with chronic exposure (Kitamura et al., 1993).

The measurement of acid-labile CS₂ in blood, presumably in the form of protein-bound dithiocarbamates, has been presented as an alternative method for evaluating acute and subacute exposures to CS₂ (McKenna and DiStefano, 1977). The analysis of acid-labile CS₂ consisted of subjecting samples to hydrolysis with acid followed by gas trapping of the released CS₂ to generate a dithiocarbamate-copper complex, which was quantified using spectrophotometry. Subsequent reports have utilized headspace analysis with gas chromatography mass spectrometry to quantify the CS₂ generated by acid hydrolysis (Brugnone et al., 1992). The practical limitations of measuring acid-labile CS₂ have most likely prevented its general implementation for monitoring exposures in the occupational setting. Because protein-bound dithiocarbamate analysis appears superior to TTCA in terms of specificity, the ability to reflect cumulative exposure, and the detection of CS₂ for longer periods after exposure has ended, we have investigated a method that consists of cleaving the thiocarbonyl function of free CS₂ and certain CS₂-generated modifications on proteins using toluene-3,4-dithiol to generate toluene trithiocarbonate. The investigation presented here evaluates the utility of the...
trithiocarbonate assay as a biomarker of internal exposure to CS₂ over acute to subchronic periods. To examine the sensitivity, dose response, and kinetics of this biomarker, rats were administered CS₂ by inhalation, ip injection, or gavage and the tolune-3,4-dithiol reactive functions generated in plasma and the intracellular compartment of erythrocytes quantified as a function of exposure level, exposure duration, and time between exposure and sample procurement. Blood was also examined following administration of a compound, captan, that produces TTCA but does not liberate CS₂, to help evaluate the relative specificity of tolune-3,4-dithiol analysis.

**MATERIALS AND METHODS**

**Chemicals.** Carbon disulfide is volatile, flammable, toxic, and a skin irritant; therefore, gloves and a fume hood should be used when handling this compound. Diazomethane (CH₃N₂) is poisonous and explosive and is therefore handled in polished glassware in a fume hood. Carbon disulfide was obtained from EM Science, Gibbstown, NJ. Captan [N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide] was obtained from Chem Service, West Chester, PA. Commercial tolune-3,4-dithiol (Aldrich Chemical Company, Milwaukee, WI) was distilled (70–80°C/0.5 Torr) and stored at ~20°C under Ar. A published procedure (Kopecky and Smejkal, 1984) was used to obtain TTCA and was modified to prepare tetrahydro-2-thioxo-2H,1,3-thiazine-4-carboxylic acid (TCA) from homocysteine (Fluka Chemical Corp., Ronkonka, NY) as described previously (Johnson et al., 1996). Corn oil was obtained from Sigma Chemical Co., St. Louis, MO.

**Animals.** This study was performed in accordance with the National Institutes of Health’s Guide for Care and Use of Laboratory Animals, and was approved by the institutional animal care committee. For the inhalation experiments, male and female Fischer 344 rats, 6–7 weeks old (Charles River Breeding Laboratories, Raleigh, NC) were used after a 2-week acclimation period. For oral (po) and intraperitoneal (ip) exposures, male and female Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC), typically weighing 200–250 g, were used and housed in a room on a diurnal light cycle with rodent Chow and water supplied ad libitum. For TTCA analysis, rats were housed individually in metabolic cages, given finely ground diet, and an inhalation chamber containing 30 mg captan, 1.2 ml 1,2 propanediol and 2.4 ml phosphate buffer. We injected diazomethane, taken to dryness, and reconstituted in ethyl acetate. We injected methanolic solution at 0.5, 50, 500, or 800 ppm for 2 weeks (6 h/day 5 days/week; n, 4, for each exposure level) were used. Aliquots of plasma were analyzed before and after storage at 4°C for 9 days.

**Captan administration.** A single blood sample (0.4 ml) was drawn from a tail vein and urine collected for 24 h prior to dosing, to determine baseline values. Captan was administered to 3 male rats (70 mg/kg) using a suspension containing 30 mg captan, 1.2 ml 1,2 propanediol and 2.4 ml phosphate buffer containing normal saline. The pH was 7.4, by gavage. Blood was obtained 24 h after dosing and analyzed for trithiocarbonate-generating compounds. Urine was collected for 24 h after dosing and analyzed for TTCA.

**Stability of protein tolune-3,4-dithiol reactive moieties.** Plasma samples obtained from male rats exposed to CS₂ at control, 50, 500, or 800 ppm for 2 weeks (6 h/day 5 days/week; n, 4, for each exposure level) were used. Aliquots of plasma were analyzed before and after storage at 4°C for 9 days.

**GC/MS measurements.** A Hewlett-Packard 5890 Series II gas chromatograph connected to a 5971A mass selective detector was used. An HP-5 column (30 m × 0.25 mm × 0.25 μm) was employed with temperature programming (70°C for 2 min, then 20°C/min to 210°C, and held). TTCA determination. Analyses were performed using a method modified from a previously published protocol (van Doorn et al., 1981). Urine was centrifuged 15 min at 5700 g and then filtered via suction through a 0.45 μm filter. The filtered urine was spiked with a constant concentration of TTCA as an internal standard and titrated to pH 2 with HCl. The urine was then extracted with ethyl acetate (3 × 2 volumes), and the extracts combined and dried over Na₂SO₄. The combined extracts were dried by evaporation and reconstituted in 1 ml methanol; 500 μl of the methanolic solution was methylated with excess diazomethane, taken to dryness, and reconstituted in ethyl acetate. We injected 0.5 μl of this onto the GC/MS and detected using multiple ion monitoring of the molecular ion at m/z 191 and the fragment at m/z 132, corresponding to the loss of the methoxy carbonyl side chain. Quantities of TTCA in urine were determined through comparison to a standard curve, and the limit of detection was 10 pmol.

**Tolune-3,4-dithiol analysis.** Blood (0.4 ml) was transferred to a 2-ml centrifuge tube containing heparin and centrifuged at 4000 g for 5 min at 4°C. The supernatant plasma was divided into one 10-μl aliquot (for protein determination using the Bradford method) and 3 50-μl aliquots (for tolune-
RESULTS

Dose response following ip and inhalation exposure. The level of toluene-3,4-dithiol reactive moieties present in hemolysate and plasma following a single ip injection of CS₂ are presented as a function of exposure level in Figure 1A. Samples obtained prior to dosing or 3 h after administering 0.04 mmol/kg CS₂ did not generate detectable levels of toluene trithiocarbonate. Greater levels of exposure resulted in detectable amounts of toluene trithiocarbonate in both plasma and hemolysate, the quantity of which demonstrated a linear dose response over the exposure range examined (plasma slope = 638.05, R² = 0.989; hemolysate slope = 40.41, R² = 0.999).

Analysis of plasma proteins from rats exposed to CS₂ by inhalation at control, 50, 500, and 800 ppm for 5 days a week, for 2 weeks produced quantities of toluene trithiocarbonate that were significantly different (p < 0.05) from the next lower exposure level. The means (n, 17; 10 male and 7 female) for controls and n, 7; 4 males and 3 females) for the 50, 500, and 800 ppm exposure groups (with standard errors are presented as a function of exposure level in Figure 1B. The results demonstrate a linear response (slope = 1.30, R² = 0.999) over the exposure levels examined.

FIG. 1. Dose response of toluene-3,4-dithiol reactive moieties in blood.

Levels of toluene trithiocarbonate generated from plasma (open squares) or hemolysate (closed squares) samples are shown as a function of CS₂ dose after ip (0, 0.04, 0.2, 1, and 5 mmol/Kg) (A) or inhalation exposure (0, 50, 500, or 800 ppm 6 h/day, 5 days a week, for 2 weeks (week days only)) (B). Values are expressed as pmol of toluene trithiocarbonate (ttc) per mg of protein and represent means ± SE (for ip injection, n, 9 for pre-dose values and n, 3 for all other exposure levels; and for inhalation n, 17 for controls and n, 7 for all other exposure levels; error bars are shown when larger than the symbols) with all samples run in triplicate.
Accumulation and elimination after a single ip injection.

The levels of toluene-3,4-dithiol reactive functional groups present in plasma and hemolysate resulting from a single ip injection of CS₂ at 1 mmol/kg are shown as a function of time in Figure 2A. The levels in hemolysate and plasma peaked within 1 and 3 h, respectively. Elimination of toluene-3,4-dithiol reactive functional groups from plasma appeared to follow a first order process (Fig. 2B) with a half-life of 11.9 h, whereas elimination from hemolysate appeared biphasic with corresponding half-lives of 1.0 and 75.0 h.

Accumulation and elimination associated with repeated subacute ip dosing. The quantities of toluene-3,4-dithiol reactive functional groups produced in hemolysate and plasma from repeated ip injection over a 5-day period are presented in Figure 3A. Plasma levels appeared to plateau after the third dose at a level approximately 16-fold greater than the hemolysate on the corresponding day. Alternatively, the amount of toluene trithiocarbonate generated from hemolysate appeared to increase over the entire exposure period. Starting from 24 h after the final dose, the elimination of toluene-3,4-dithiol reactive functional groups demonstrated first order kinetics with estimated half-lives for plasma and hemolysate of 20.5 and 129.6 h, respectively (Fig. 3B).

Accumulation and elimination associated with repeated subchronic po dosing. Analysis of blood proteins with repeated dosing over subchronic periods revealed a cumulative dose response for both blood compartments in the end of the week samples obtained over the 5-week period examined (Fig. 4A). In comparison, the values obtained on Monday morning approximately 72 h after the previous dose, although still detectable, did not appear to reflect sequential increases. Levels of toluene trithiocarbonate generated from samples as a function of time after the cessation of exposure are presented in Figure 4B. The estimated elimination half-life for the terminal phase in plasma was 19.3 h and for hemolysate was 693.0 h.

Administration of captan. No trithiocarbonate was generated from hemolysate or plasma prepared from the samples obtained prior to captan administration or at 3 or 24 h after administration of captan. TTCA was not detected in the 24-h urine sample collected before administration, and the mean (SE) 24-h urinary excretion of TTCA after dosing with captan was 660 (235) nmol.

Stability of toluene-3,4-dithiol reactive moieties. After storage at 4°C for 9 days, the quantity of toluene trithiocarbonate generated from plasma samples was reduced by 14.9 ± 0.69% in the samples obtained from exposed rats. No toluene trithiocarbonate was generated from control samples before or after storage.

DISCUSSION

The method of exposure assessment presented here utilizes the facile and quantitative reaction between the electrophilic carbon of a thiocarbonyl moiety and the vicinal sulfhydryl groups of toluene-3,4-dithiol (Scheme I). The resulting product is a non-protein bound toluene trithiocarbonate amenable to separation and quantification using HPLC with UV detection. Previously this reaction was used to analyze for isothiocyanates present in plant extracts (Zhang et al., 1992, 1996). Those prior studies showed
that benzenedithiol yielded quantitative cyclocondensation products with several thiocarbonyl compounds including dithiocarbamates, polythiocarbamates, xanthates, N-aromatic thioureas, and CS$_2$, suggesting that more than one type of CS$_2$-generated species may be detected by the reagent in blood. Reviewing the recognized interactions of CS$_2$ with protein amino functions under physiological conditions (Valentine et al., 1992, 1995) (Scheme II), 4 types of toluene-3,4-dithiol reactive species are apparent: dithiocarbamates, isothiocyanate intermediates, and the disulfide and dithiocarbamate ester cross-linking structures. Because the

![Graph 1](image1)

![Graph 2](image2)

**FIG. 3.** Accumulation and elimination of toluene-3,4-dithiol reactive functions during and after repeated ip exposure at 24-h intervals over 5 days. (A) Levels of toluene trithiocarbonate generated from plasma (open squares) and hemolysate (closed squares) samples obtained on days 1, 2, and 4, immediately prior to daily ip administration of CS$_2$ (1.5 mmol/kg/day) and 24 h after the fifth dose, are shown. Elimination of toluene-3,4-dithiol reactive functions beginning 24 h after the final dose (B) approximated a single exponential process for plasma (open squares) and hemolysate (closed squares), with half times of 20.5 h and 129.6 h, respectively. Values are expressed as pmol of toluene trithiocarbonate (ttc) per mg of protein and represent means ± SE (n, 4) with all samples run in triplicate.

**FIG. 4.** Accumulation and elimination of toluene-3,4-dithiol reactive functions during and after a 5 week po exposure. (A) Levels of toluene trithiocarbonate generated from plasma (open squares) and hemolysate (closed squares) samples obtained from rats administered CS$_2$ po daily Monday through Friday at 0.5 mmol/kg/day for 5 weeks. Samples were acquired on Monday immediately prior to dosing (starting day 0) and 3 h after dosing on Friday (starting with day 5). (B) Levels of toluene-3,4-dithiol reactive functions in plasma (open squares) and hemolysate (closed squares) are shown as a function of time beginning 3 h after the final oral dose of CS$_2$. Estimated elimination half times were 19.3 h and 693.0 h for plasma and hemolysate, respectively. Values are expressed as pmol of toluene trithiocarbonate (ttc) per mg of protein and represent means ± SE (n, 5) with all samples run in triplicate.
cyclocondensation reaction does not proceed with N-alkyl thioureas (data not shown) there is no contribution expected from thiourea-protein cross-linking structures. Potentially, CS₂ can also react with sulfhydryl groups to generate a protein-bound trithiocarbonate moiety that may contribute to the generation of toluene trithiocarbonate (Valentine et al., 1992). Because of the relatively small number of free sulfhydryl groups compared to amino groups for most proteins, the anticipated contribution from sulfhydryl modification would appear minimal. A possible exception may arise for hemolysate, in which the high concentration of sulfhydryls associated with globin and reduced glutathione may contribute to a non-negligible quantity of intracellular CS₂-generated trithiocarbonate. Similarly, xanthates generated on protein hydroxy functions are not expected to contribute significantly in this study, due to the relatively low reactivity of hydroxyl groups for nucleophilic addition to CS₂ at neutral pH (Valentine et al., 1992). Based upon data obtained previously in vitro (Valentine et al., 1992, 1995) and from measurements of acid-labile forms of CS₂ produced in vivo (Lam and DiStefano, 1982, 1986; Lam et al., 1986), it is expected that the initial dithiocarbamate and free CS₂ are the major dithiol reactive moieties in blood, with the ratio of these 2 chemical species dependent upon the time interval after exposure and the blood compartment analyzed.

Several advantages of dithiol analysis compared to existing methods for CS₂-exposure assessment are apparent from the present study. Although similar information can be obtained from the measurement of acid-labile CS₂, dithiol analysis requires less sample, detects more persistent protein modifications in hemolysate, and is more amenable to batch analysis with automation. Relative to headspace analysis of free CS₂, sample handling is much less critical for dithiol analysis. The levels of dithiol reactive functions in blood also display a linear response over a very wide range of exposure levels, after ip or inhalation exposure. This suggests a greater dynamic range than the analysis of free CS₂ for which no increases above the 500-ppm level were observed (Moorman et al., 1998). Similarly, although contradictory results have been reported (Cox et al., 1996), studies have provided evidence that the urinary excretion of TTCA is saturable at high levels of CS₂ exposure and dependent upon levels of hepatic glutathione (Kivisto et al., 1995; Moorman et al., 1998). Regarding specificity, the present findings support the ability of dithiol analysis to dis-
caminate between CS$_2$ and captan, a non-CS$_2$-generating compound that is known to elevate excretion of TTCA (DeBaun et al., 1974). Probably of greatest significance in terms of risk assessment is the potential of dithiol analysis to reflect cumulative exposures, and to reveal exposures for greater periods of time after cessation of exposure than can the measurement of free CS$_2$ or urinary TTCA.

Substantial differences were observed for the plasma and hemolysate compartments. The total quantity of dithiol reactive functions in plasma exceeded hemolysate except for the very early and very late time points after exposure. This time dependence appeared to result from the fact that removal of dithiol reactive functions from plasma followed a single exponential decrease, whereas removal from hemolysate appeared biphasic. One potential explanation to account for the biphasic behavior observed for hemolysate may arise from the ability of CS$_2$ to exist in free and protein-bound forms. The half-life calculated here for the initial rapid phase in hemolysate corresponds closely to the terminal elimination half-lives reported previously for free CS$_2$ from blood (Lam and DiStefano, 1982; Moorman et al., 1998). Furthermore, previous studies have demonstrated that greater than 90% of free CS$_2$ in blood resides within the red cell (Lam et al., 1986). Thus, the initial phase observed here for the hemolysate appears consistent with the elimination of free CS$_2$ from blood, with the second slower phase corresponding to the removal of CS$_2$-mediated protein modifications. Also consistent with previously reported results, the sampling times used in the present study precluded the detection of the more rapid phase of free CS$_2$ distribution that is on the order of 8 min. Although the elimination of dithiol reactive functions from plasma appeared too slow to correspond to free CS$_2$, it was more rapid than the terminal phase observed for hemolysate, a relationship that may prove useful as a tool to estimate the interval between exposure and sample procurement. At relatively short intervals, plasma levels will exceed hemolysate, and with progressively longer periods this ratio will diminish until only hemolysate samples remain positive.

Dithiol analysis detects several CS$_2$-derived protein modifications as well as free CS$_2$, each with the potential to be eliminated through independent processes. The observed rate of removal for each dithiol reactive species is expected to be determined by its chemical stability and biological life of the modified protein. Therefore, the overall observed elimination of dithiol reactive moieties from blood is complex and probably a combination of both first order and zero order processes, the relative contributions of which depend upon the time of sampling and blood compartment being examined. Among the 3 major anticipated dithiol reactive species, free CS$_2$ does not exist to an appreciable extent in plasma and is removed rapidly from the red cell compartment. For protein-associated dithiocarbamates, regeneration of CS$_2$ and parent amine, with subsequent removal of CS$_2$ likely predominates in hemolysate and possibly plasma. However, removal of albumin, which has a circulating half-life of approximately 2 days, may also contribute to the observed elimination of dithiocarbamate adducts from plasma. The stability of dithiocarbamate esters provides for accumulation over a more extended period and suggests that its elimination is more dependent upon the removal of the associated proteins. For hemoglobin, this is not a random process, in that erythrocytes are removed through senescence with a lifespan on the order of 60 days in the rat. Because stable dithiocarbamate esters would not have been detected by previous methods used to measure acid-labile CS$_2$, this structure may provide an explanation to account for the longer elimination rates observed for dithiol reactive functions in hemolysate relative to those reported for acid-labile CS$_2$ in blood (Lam and DiStefano, 1982). Additionally, continued accumulation of dithiocarbamate ester cross-links on erythrocyte proteins with prolonged exposure to CS$_2$ has been supported by previous investigations (Valentine et al., 1993, 1998) and may account for the relationship of elimination times to exposure duration observed here. This interpretation must be viewed with caution though, in that the experimental conditions used prevent an unequivocal interpretation and the analytical treatment is approximate, but because of the potential utility of such a relationship, this possibility warrants further investigation.

In the subchronic experiment, samples collected after the final exposure each week exhibited a cumulative response over the entire exposure period, suggesting that end of shift samples obtained on Friday may provide the greatest sensitivity for detection in the occupational setting. In contrast, predose samples obtained on Monday morning appeared to peak at 1 and 3 weeks for plasma and hemolysate, respectively. These data support the potential of dithiol analysis for evaluating cumulative exposures but, because single daily dose administration of CS$_2$ is a poor model for the level of protein modifications produced by sustained inhalation of CS$_2$ (Valentine et al., 1997), investigations using inhalation exposures are required to more clearly define the utility of toluene-3,4-dithiol analysis to integrate cumulative exposures in the workplace.

The results presented here support the utility of measuring dithiol reactive components in blood for assessing internal exposure to CS$_2$. This method may also be applicable to monitoring exposure to dithiocarbamates, which can liberate CS$_2$ in vivo in sufficient quantities to produce neurotoxicity (Johnson et al., 1996, 1998). If plasma and hemolysate samples are not prepared in triplicate, and multiple HPLC injections from a single preparation used instead, only a small volume of blood is needed and may be obtainable from a finger stick, thereby eliminating the need for venipuncture. Although the detection of multiple CS$_2$-mediated protein modifications together with free CS$_2$ complicates the interpretation of the chemical species being quantified and the observed elimination rates, it does contribute to the sensitivity of the method for detecting exposure. Additionally, if required, this complication can be minimized by sampling at later time points or removing
free CS₂ by filtration. Further studies are required to determine the sensitivity, dose response, and kinetics associated with inhalation exposures within the range experienced in the workplace and to investigate the formation of dithiol reactive moieties in human blood. The success of previous studies to detect exposures in the 5 to 40 ppm range using acid-labile CS₂ (Lam and DiStefano, 1983) and the known reactions of CS₂ with proteins both suggest that this can be accomplished. Additional studies should also be directed at determining the relative contributions and associated elimination rates of the individual dithiol reactive chemical species produced by CS₂ as a function of exposure duration.

Studies have shown the ability of CS₂-mediated protein cross-linking in erythrocyte-associated proteins to provide mechanistically based assessments of CS₂ exposure, although the sensitivity and response time of these events do not appear appropriate for evaluating acute exposures (Erve et al., 1998; Valentine et al., 1997). Therefore, dithiol analysis in conjunction with erythrocyte protein cross-linking may provide the ability to characterize the internal dose of CS₂ resulting from acute through chronic periods, and provide insight into the level of protein covalent modification and cross-linking present within the nervous system. The benefit of this methodology could be realized in the development of intervention strategies directed toward identifying and removing individuals from neurotoxic levels of CS₂ prior to the development of neurological deficits.

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REFERENCES


